Insights into the red algae and eukaryotic evolution from the genome of *Porphyra umbilicalis* (Bangiophyceae, Rhodophyta)

Susan H. Brawley[a,1], Nicolas A. Blouin[a,b], Elizabeth Ficko-Blean[c], Glen L. Wheeler[d], Martin Lohr[e], Holly V. Goodson[f], Jerry W. Jenkins[g,h], Crysten E. Blaby-Haas[i], Katherine E. Helliwell[d,j], Cheong Xin Chan[k,l], Tara N. Marriage[m], Debashish Bhattacharya[n], Anita S. Klein[o], Yacine Badis[p], Juliet Brodie[q], Yuanyu Cao[o,2], Jonas Collén[c], Simon M. Dittami[c], Claire M. M. Gachon[p], Beverley R. Green[r], Steven J. Karpowicz[s], Jay W. Kim[t], Ulrich Johan Kudahl[j], Senjie Lin[u], Gurvan Michel[c], Maria Mittag[v], Bradley J. S. C. Olson[m], Jasmyn L. Pangilinan[h], Yi Peng[h], Huan Qiu[n], Shengqiang Shu[h], John T. Singer[w], Alison G. Smith[j], Brittany N. Sprecher[u], Volker Wagner[v], Wenfei Wang[x], Zhi-Yong Wang[y], Juying Yan[h], Charles Yarish[z], Simone Zäuner-Riek[aa], Yunyun Zhuang[u,3], Yong Zou[v], Erika A. Lindquist[h], Jane Grimwood[g,h], Kerrie W. Barry[h], Daniel S. Rokhsar[h], Jeremy Schmutz[g,h], John W. Stiller[bb], Arthur R. Grossman[y], and Simon E. Prochnik[h]

[a]School of Marine Sciences, University of Maine, Orono, ME 04469; [b]Department of Molecular Biology, University of Wyoming, Laramie, WY 82071; [c]Sorbonne Universités, Université Pierre and Marie Curie Paris 06, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, 29688 Roscoff, France; [d]Marine Biological Association of the United Kingdom, Plymouth, PL1 2PB, United Kingdom; [e]Institut für Molekulare Physiologie, Pflanzenbiochemie, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany; [f]Department of Chemistry & Biochemistry, University of Notre Dame, South Bend, IN 46556; [g]HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806; [h]Department of Energy, Joint Genome Institute, Walnut Creek, CA 94598; [i]Biology Department, Brookhaven National Laboratory, Upton, NY 11973; [j]Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom; [k]Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD 4072, Australia; [l]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD 4072, Australia; [m]Division of Biology, Kansas State University, Manhattan, KS 66506; [n]Department of Ecology, Evolution & Natural Resources, Rutgers University, New Brunswick, NJ 08901; [o]Department of Biological Sciences, University of New Hampshire, Durham, NH 03824; [p]Scottish Association for Marine Sciences, Scottish Marine Institute, Oban PA37 1QA, United Kingdom; [q]Natural History Museum, Department of Life Sciences, London SW7 5BD, United Kingdom; [r]Department of Botany, University of British Columbia, Vancouver BC, Canada V6T 1Z4; [s]Tau Biosciences LLC, Edmond, OK 73003; [t]Department of Biomolecular Engineering, University of California, Santa Cruz, CA 95064; [u]Department of Marine Sciences, University of Connecticut, Groton, CT 06340; [v]Institut für Allgemeine Botanik und Pflanzenphysiologie, Friedrich-Schiller-Universität Jena, 07743 Jena, Germany; [w]Department of Molecular & Biomedical Science, University of Maine, Orono, ME 04469; [x]Basic Forestry and Proteomics Research Center, HIST, Fujian Agriculture and Forestry University, Fuzhou 350002, China; [y]Department of Plant Science, Carnegie Institution for Science, Stanford, CA 94305; [z]Department of Ecology & Evolutionary Biology, University of Connecticut, Stamford, CT 06901; [aa]Institute of Molecular Physiology and Biotechnology, University of Bonn, 53115 Bonn, Germany; and [bb]Department of Biology, East Carolina University, Greenville, NC 27858

Edited by Stephen R. Palumbi, Stanford University, Pacific Grove, CA, and approved June 6, 2017 (received for review February 22, 2017)

Porphyra umbilicalis **(laver) belongs to an ancient group of red algae (Bangiophyceae), is harvested for human food, and thrives in the harsh conditions of the upper intertidal zone. Here we present the 87.7-Mbp haploid *Porphyra* genome (65.8% G + C content, 13,125 gene loci) and elucidate traits that inform our understanding of the biology of red algae as one of the few multicellular eukaryotic lineages. Novel features of the *Porphyra* genome shared by other red algae relate to the cytoskeleton, calcium signaling, the cell cycle, and stress-tolerance mechanisms including photoprotection. Cytoskeletal motor proteins in *Porphyra* are restricted to a small set of kinesins that appear to be the only universal cytoskeletal motors within the red algae. Dynein motors are absent, and most red algae, including *Porphyra*, lack myosin. This surprisingly minimal cytoskeleton offers a potential explanation for why red algal cells and multicellular structures are more limited in size than in most multicellular lineages. Additional discoveries further relating to the stress tolerance of bangiophytes include ancestral enzymes for sulfation of the hydrophilic galactan-rich cell wall, evidence for mannan synthesis that originated before the divergence of green and red algae, and a high capacity for nutrient uptake. Our analyses provide a comprehensive understanding of the red algae, which are both commercially important and have played a major role in the evolution of other algal groups through secondary endosymbioses.**

cytoskeleton | calcium-signaling | carbohydrate-active enzymes | stress tolerance | vitamin B_{12}

The red algae are one of the founding groups of photosynthetic eukaryotes (Archaeplastida) and among the few multicellular lineages within Eukarya. A red algal plastid, acquired through secondary endosymbiosis, supports carbon fixation, fatty acid synthesis, and other metabolic needs in many other algal groups in ways that are consequential. For example, diatoms and haptophytes have strong biogeochemical effects; apicomplexans cause human disease (e.g., malaria); and dinoflagellates include both coral symbionts and toxin-producing "red tides" (1). The evolutionary processes that produced the Archaeplastida and secondary algal lineages remain under investigation (2–5), but it is clear that both nuclear and plastid genes from the ancestral red algae have contributed dramatically to broader eukaryotic evolution and diversity. Consequently, the imprint of red algal metabolism on the Earth's climate system, aquatic foodwebs, and

Author contributions: S.H.B., N.A.B., D.B., S.L., C.Y., K.W.B., D.S.R., J.S., J.W.S., A.R.G., and S.E.P. designed research; S.H.B., N.A.B., E.F.-B., G.L.W., M.L., H.V.G., J.W.J., C.E.B.-H., K.E.H., C.X.C., T.N.M., D.B., A.S.K., Y.B., J.B., Y.C., J.C., S.M.D., C.M.M.G., B.R.G., S.J.K., J.W.K., U.J.K., S.L., G.M., M.M., B.J.S.C.O., J.L.P., Y.P., H.Q., S.S., A.G.S., B.N.S., V.W., W.W., Z.-Y.W., J.Y., S.Z.-R., Y. Zou, E.A.L., J.G., K.W.B., D.S.R., J.S., J.W.S., A.R.G., and S.E.P. performed research; S.H.B., N.A.B., E.F.-B., G.L.W., M.L., H.V.G., J.W.J., C.E.B.-H., K.E.H., C.X.C., D.B., S.M.D., C.M.M.G., S.J.K., G.M., H.Q., A.G.S., J.S., J.W.S., and S.E.P. contributed new reagents/analytic tools; S.H.B., N.A.B., E.F.-B., G.L.W., M.L., H.V.G., J.W.J., C.E.B.-H., K.E.H., C.X.C., T.N.M., D.B., A.S.K., Y.B., J.B., Y.C., J.C., S.M.D., C.M.M.G., B.R.G., S.J.K., J.W.K., U.J.K., S.L., G.M., M.M., B.J.S.C.O., H.Q., A.G.S., B.N.S., V.W., W.W., Z.-Y.W., S.Z.-R., Y. Zou, J.S., J.W.S., A.R.G., and S.E.P. analyzed data; S.H.B., N.A.B., S.L., J.T.S., C.Y., Y. Zhuang, and A.R.G. prepared samples; and S.H.B., N.A.B., E.F.-B., G.L.W., M.L., H.V.G., J.W.J., C.E.B.-H., K.E.H., C.X.C., T.N.M., D.B., A.S.K., Y.B., J.B., Y.C., J.C., S.M.D., C.M.M.G., B.R.G., S.J.K., J.W.K., U.J.K., S.L., G.M., M.M., B.J.S.C.O., H.Q., J.T.S., A.G.S., B.N.S., V.W., W.W., Z.-Y.W., C.Y., S.Z.-R., Y. Zhuang, Y. Zou, J.W.S., A.R.G., and S.E.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The Whole Genome Shotgun project has been deposited at the DNA Data Bank of Japan/European Nucleotide Archive/GenBank (accession no. MXAK00000000). The accession no. for the chloroplast genome is MF385003.

[1]To whom correspondence should be addressed. Email: Brawley@maine.edu.

[2]Present address: Genetics Program, University of New Hampshire, Durham, NH 03824.

[3]Present address: College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1703088114/-/DCSupplemental.

Significance

Fossil evidence shows that red algae (Rhodophyta) are one of the most ancient multicellular lineages. Their ecological, evolutionary, and commercial importance notwithstanding, few red algal nuclear genomes have been sequenced. Our analyses of the *Porphyra umbilicalis* genome provide insights into how this macrophyte thrives in the stressful intertidal zone and into the basis for its nutritional value as human food. Many of the novel traits (e.g., cytoskeletal organization, calcium signaling pathways) we find encoded in the *Porphyra* genome are extended to other red algal genomes, and our unexpected findings offer a potential explanation for why the red algae are constrained to small stature relative to other multicellular lineages.

human health is immense. Moreover, the oldest taxonomically resolved multicellular eukaryote in the fossil record (1.2 Ga) is the bangiophyte red alga *Bangiomorpha*, which closely resembles the extant marine alga *Bangia* (6). As is typical of most bangiophytes, *Porphyra* grows in one of Earth's most physically stressful habitats, the intertidal zone, where organisms are exposed to daily and seasonally fluctuating temperatures, high levels of irradiance (including UV), and severe osmotic stress and desiccation. *Porphyra* and its ancestors have competed successfully in this dynamic and severe environment for over a billion years, through numerous changes in climate and mass extinctions.

Here we describe the genome of *Porphyra umbilicalis*. Examination of the *Porphyra* genome and complete genomes of other red algae [*Chondrus crispus* (7), *Cyanidioschyzon merolae* (8), *Galdieria sulphuraria* (9), *Porphyridium purpureum* (10), *Pyropia yezoensis* (11)] revealed numerous additional differences between the red algae and other eukaryotic lineages, including a reduced complement of motor proteins, unique signaling molecules, and augmented stress tolerance mechanisms, especially in *Porphyra*.

Results and Discussion

Genomic Analysis. An 87.7-Mbp assembly of the *P. umbilicalis* (hereafter, *Porphyra*) nuclear genome was generated from PacBio whole-genome shotgun sequencing, with insertions and deletions corrected using Illumina whole-genome shotgun reads (*SI Appendix*, *Methods*). The *Porphyra* genome has a substantial repeat component (43.9%) for a compact genome, with the most common repeat classes being DNA (15.5 Mbp) and LTR (14.9 Mbp) elements (*SI Appendix*, Table S5). Gene models were predicted at 13,125 loci using de novo gene prediction algorithms supported by evidence from protein homology and expression data (*SI Appendix*, Table S6). A typical gene has ~two exons, implying abundant splicing for a red alga; however, only 235 alternative splice-forms were identified from expressed sequence tag coverage of genes (*SI Appendix*, Table S6). Overall, the genome is 65.8% G+C, but protein-coding regions average 72.9% and reach up to 94% G+C (*SI Appendix*, Fig. S7). Nearly 98% of the sequenced transcripts (expressed sequence tags) can be mapped to the genome assembly, and we identified complete complements of genes encoding RNA polymerase subunits and all other conserved proteins involved in transcription, translation, and DNA synthesis (*SI Appendix*, Table S10), suggesting that the genome is nearly complete.

Phylogenomic analysis (12) of the red algae (Rhodophyta) distinguishes a class (Cyanidiophyceae) of extremophilic unicellular species and two sister clades of mesophilic species, which we refer to here as the SCRP (Stylonematophyceae, Compsopogonophyceae, Rhodellophyceae, Porphyridiophyceae) and the BF (Bangiophyceae, Florideophyceae) (*SI Appendix*, Fig. S11). The SCRP clade contains unicells, microscopic filaments, and microscopic blades, whereas the BF clade holds macrophytes ("seaweeds") that comprise the majority of described species (13). Phylogenomic comparisons of Bangiophyceae (e.g., *P. umbilicalis*, *P. yezoensis*) and Florideophyceae (e.g., *C. crispus*, *Calliarthron tuberculosum*) suggest that these two red algal classes are highly diverged (*SI Appendix*, Fig. S12). The absence of some pathways and genes from red algae is likely because of genomic reduction in the red algal ancestor (4), and we confirmed that *Porphyra* lacks genes described previously as lost in other red algae, including those encoding enzymes of the glycosyl-phosphatidylinositol (GPI) anchor biosynthesis pathway (Kyoto Encyclopedia of Genes and Genomes map00563, 22 genes), autophagy proteins (KO pathway ko04140, 17 genes), and most flagellar proteins (4).

Cytoskeleton. The cytoskeleton of red algae is poorly characterized, despite the long-recognized absence of flagella from the red algae (14, 15). Nuclear-associated organelles that lack centrioles appear to organize the mitotic spindle (1), and freeze-substitution reveals cytoplasmic microtubules and bundles of actin microfilaments (16). Cytoskeletal inhibitors and fluorescent probes (e.g., FITC-phalloidin for microfilaments) demonstrate that actin microfilaments form cortical rings during cytokinesis and during sperm/egg fusion, ensheath migrating secretory vesicles and organelles, and are prominently labeled in amoeboid red algal spores (17–21). Certainly the composition of the red algal cytoskeleton must determine many of the capabilities and limitations of red algae because of the fundamental roles the cytoskeleton plays in intracellular transport, secretion of cell wall materials, regulation of cell size and shape, and responses to developmental and environmental signals that influence cell polarity and complex tissue development in many eukaryotes (22, 23). Here we report that *Porphyra* and other red algae have significantly reduced cytoskeletons and consider the consequences for size and complexity.

We identified four closely related actin genes in *Porphyra*, as well as the chromatin remodeling, actin-related protein Arp4, but no other Arp proteins (*SI Appendix*, Table S15). The lack of the dynactin complex Arp1 is consistent with loss of a dynein motor, but the general absence of Arp2/Arp3 from red algae is surprising. In eukaryotes, Arp2/3 nucleates the formation of branched microfilaments that mediate amoeboid motion (22), which is observed in many types of red algal spores, including *Porphyra* neutral spores (24) and *Pyropia pulchella* archeospores (21). How these spores move without Arp2/3 is an intriguing question; perhaps they rapidly polymerize microfilaments with the aid of other nucleating machinery, such as formins (22), which are present. However, although formins in many animals, fungi, and plants are members of expanded gene families that have been differentiated to support processes required to build complex morphologies (e.g., polarized tip growth and cell plate orientation) (25, 26), *Porphyra* has only two formins (*SI Appendix*, Table S15). In addition to formins, *Porphyra* and other red algae (*SI Appendix*, Tables S15 and S16) contain profilin, which interacts with formins; cofilin, a key depolymerizing factor; and severin, which cuts microfilaments to promote remodeling. However, we did not find other well-conserved, widely distributed actin-modifying proteins (e.g., WASP/WAVE, CapZ, fimbrin) in *Porphyra*, and few if any convincing homologs in other red algae (*SI Appendix*, Table S16). The most striking limitation to microfilament-mediated phenomena in *Porphyra* and most other red algae is the absence of myosin. Myosin genes were not detected in any of the available genomes of the BF clade (Fig. 1), and only nonspecific (27) myosin inhibitors (2,3-Butanedione monoxime) were used previously (e.g., ref. 21) to infer myosin activity. We do find that the single myosin annotated previously (9) in the extremophile *Galdieria*, but absent from *Cyanidioschyzon* (8), is also found in three classes of the SCRP clade, but not in Porphyridiophyceae (Fig. 1 and *SI Appendix*, Fig. S17).

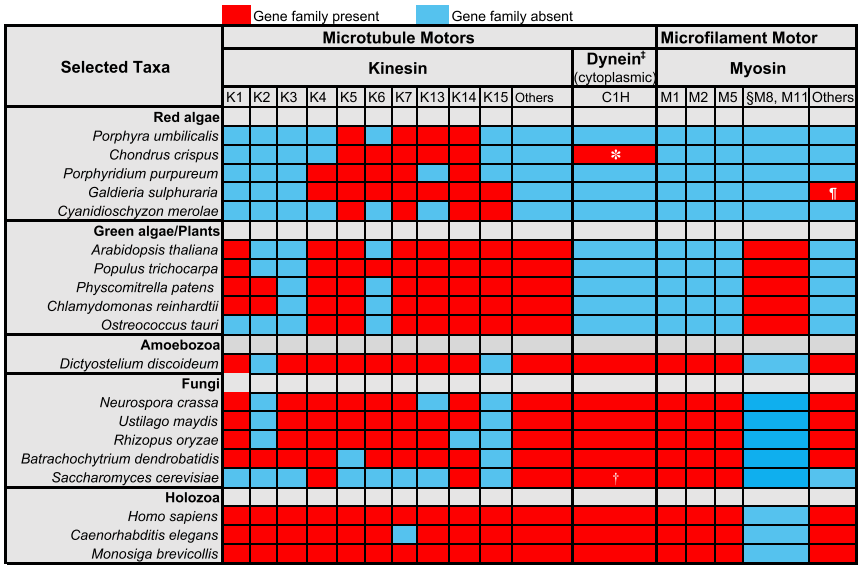

Fig. 1. Distribution of cytoskeletal motor proteins (kinesin, cytoplasmic dynein, and myosin) in red algae compared with other select groups of Eukarya, showing the reduced cytoskeletal capacity of red algal cells. Standard motor family nomenclatures for dyneins (104), kinesins (105), and myosins (106) are used; for example, kinesins K1, K2, and K3 are cytoskeletal motors that move organelles and vesicles in many eukaryotes. Footnotes: *This may be a contaminating sequence. †The *Saccharomyces* dynein complex has lost much of the functionality found in other organisms (104). ‡Additional, flagellar-related dynein HC are not shown and are absent in red algae and many plants. §Myosins M8 and M11 are vesicle transporters in plants. ¶*Galdieria* and SCR members of the SCRP clade (*SI Appendix*, Fig. S11) have a myosin similar to myosin 27 of apicomplexans.

We found that the *Porphyra* genome encodes the expected α- and β-tubulin proteins, as well as some proteins related to tubulin folding (e.g., *Porphyra* contains cofactors B and D, but not A or E) and microtubule nucleation (e.g., γ-tubulin, γ-complex proteins) (*SI Appendix*, Tables S15 and S16). However, many of the expected tubulin regulatory proteins that are widely distributed across eukaryotes and even found in other red algae are missing. For example, *Porphyra* contains EB1 and Mor1/XMAP215, two highly conserved proteins of the MT plus-end tracking (+TIP) complex, but the +TIP CLASP and the cross-linker MAP65 appear absent, even though these highly conserved genes are present in other red algae (*SI Appendix*, Table S16). Unknown as yet is whether *Porphyra* simply lacks these activities, or has recruited other proteins to fill their roles. *Porphyra* and other red algae lost flagellar and cytoplasmic dynein motors, intermediate chains, and most light chains. A dynein heavy chain reported from *C. crispus* might be from a contaminant or horizontal gene transfer (*SI Appendix*, Table S16). *Porphyra* and other red algae retain a particular light chain that is also conserved in flowering plants, which independently lost flagellar motility (*SI Appendix*, Table S16). This dynein light chain is expressed under abiotic stress and phytohormone treatments in plants (28); thus, it may have a role in the stress tolerance of *Porphyra*. In contrast to the loss of the dynein motor, *Porphyra* does have representatives of several kinesin motor subfamilies, specifically kinesins 5, 7, and 14, which are expected to be involved in spindle assembly, kinetochore function, and regulation of microtubule dynamics, respectively (Fig. 1 and *SI Appendix*, Fig. S18 and Tables S15 and S16). *Porphyra* also contains three divergent kinesins that usually group with the mitotic motor kinesin 13. However, classic vesicle transport motors (kinesin subfamilies 1, 2, and 3) appear to be absent in *Porphyra*, which is particularly surprising considering the apparent loss of myosins and dyneins.

Given the surprising paucity of motors, how do *Porphyra* cells accomplish intracellular transport of membranes or other cargo? One answer may be provided by the observation that the "mitotic" motor kinesin 14 (present in all sequenced red algae, see Fig. 1) can act as a minus-end directed transporter in land plants (reviewed by refs. 29 and 30). In addition, a few red algae do contain members of the kinesin 4 subfamily (Fig. 1), which are reported to be plus-end directed microtubule vesicle motors in plants (31). However, *Porphyra* and most other sequenced red algae lack this protein. Thus, unless kinesin 5 or kinesin 7 has unexpected functionality, *Porphyra* and the majority of red algae (BF clade) would also apparently lack a plus-end directed kinesin-microtubule motor system. Taken together, these data show that the paucity of motors explains the absence of cell streaming from red algae including *Porphyra*.

One counterpoint to the frequent absence of near-ubiquitous cytoskeletal proteins is that *Porphyra* does have two septins (*SI Appendix*, Tables S15 and S16), filament-forming proteins that are involved in cytokinesis, cell polarity, and membrane remodeling (22, 23). Regardless, our overall analysis of *Porphyra* and other red algae with sequenced nuclear genomes [*Chondrus* (7), *Cyanidioschyzon* (8), *Galdieria* (9), *Porphyridium* (10), *Pyropia* (11)] indicates that the red algal cytoskeleton lacks the complexity and diversity of cytoskeletal elements present in other multicellular lineages (Fig. 1). Although it is possible that regulatory proteins are simply too divergent to recognize, the paucity of motors is especially apparent. We suggest that this observation could help to explain long-standing questions about morphological evolution in the red algae, including the lack of parenchyma in these organisms. Compared with other multicellular lineages (green algae/plants, brown algae, fungi, animals), the abilities to form large cells and large multicellular structures appear to be limited in the red algae. For example, the largest cells in *Porphyra* are found in its holdfast, which is composed of thousands of thread-like, slow-growing rhizoid cells that are millimeters long, and some species of *Griffithsia* (32), which is a subtidal florideophyte, have cells ∼2-mm long. In contrast, large cells filling special niches or functions evolved in multiple freshwater and marine green algae, including coenocytes (1), as germinating pollen tubes in land plants (33), as sporangiophores in fungi (34), as nerve cells in animals (35), and as sieve elements in brown algae (36). Maintenance of large cells would be expected

to require vigorous multidirectional intracellular transport, which seems unlikely with a motor repertoire as limited as that seen in *Porphyra* and the other red algae with sequenced genomes (Fig. 1).

Similarly, brown algae (46-m kelps) (36), animals, and plants assemble large, complex 3D body plans with true parenchyma, but multicellular forms of red algae mostly consist of simple filaments or filaments interwoven and tacked together by secondary pit plugs (1) (i.e., pseudoparenchyma). Red algae are usually ≤50-cm long and only a few species reach 2 m in length (36, 37). Fungi cannot make parenchyma, but saprophytic mycelia grow to ≥50-m length (38). Failure of the red algae to form large multicellular structures is not straightforwardly attributed to cytoskeletal limitations; however, plants, which retain two motors (myosin, kinesin) despite their independent loss of dynein, suffer serious stunting and other developmental abnormalities following gene knockouts of myosins (23, 39). Analysis of major transcriptional and developmental regulators does not offer a compelling explanation for why red algae have failed to evolve tissues comparable to those in brown algae, plants, and animals (40). Taken together, our comparative analysis of the genomes of *Porphyra* and other red algae leads us to speculate that the small number of cytoskeletal elements in red algae compared with those in other multicellular lineages (Fig. 1) has constrained the ability of red algae to develop larger, more complex cells and multicellular structures.

Stress. The ecological success of *Porphyra* and many of the closely related bangiophyceans (37, 41) in the intertidal zone suggests that these species developed cellular mechanisms to cope with this harsh environment. In particular, *Porphyra* grows from the mid-to-high intertidal zone, where it is routinely exposed during daily low tides to high light, desiccation, and extreme fluctuations in temperature and salinity. Blades can lose up to 95% of their water on some days, but are metabolically active as soon as they are rehydrated by the rising tide (24). Here we infer novel adaptations to cope with these stresses.

Photoprotection. Light is required for photosynthesis, but severe cellular damage can result from exposure of photosynthetic organisms to the high levels of light (visible and UV) that are present in the mid-to-high intertidal zone where most bangiophycean algae, including *Porphyra*, grow. *Porphyra* has the same complement of genes (*SI Appendix*, Table S19) to carry out photosynthesis as other red algae, but exhibits a notable set of photoprotection strategies. Among the 13 genes encoding chlorophyll a-binding light-harvesting complex proteins are two "RedCap" genes (42), which may be involved in reorganization of the photosynthetic antenna during the shift from darkness to light (43). *Porphyra* also has 11 genes encoding "high light-induced" or "one-helix" proteins (here OHPs) that have essential roles for photoacclimation and cell viability under stressful environmental conditions (43, 44). Mechanistically, OHPs may regulate chlorophyll and tetrapyrrole biosynthesis, stabilize photosystem I (PSI), bind free chlorophyll or chlorophyll-breakdown products from damaged PSII complexes during the damage/repair cycle, or bind carotenoids that dissipate excess absorbed light energy. In contrast to the 11 *Porphyra* OHPs (*SI Appendix*, Table S19), we found 4 OHPs in *Chondrus*, which experiences less drying and light stress because of its low intertidal/subtidal habitat, 6 in *P. yezoensis*, 7 in *Porphyridium*, and only 1 OHP in *Cyanidioschyzon*, which inhabits a stable hotspring environment (*SI Appendix, Photosynthesis, Photoprotection, Stress Genes*). More analysis is needed, but the putative gene family expansion in *Porphyra* suggests positive selection for increased gene dosage. *Porphyra* was one of the first organisms where quenching of excess excitation energy in response to desiccation stress was observed (44), but the molecular mechanisms responsible for this quenching in red algae remain unclear.

Porphyra encodes genes for catalases and peroxidases, as well as the biosynthesis of numerous antioxidants, such as ascorbic acid (vitamin C) and tocopherol (vitamin E) (*SI Appendix*, Tables S20–S22). When overexcitation of photosynthetic electron transport occurs and reactive oxygen is generated, catalase detoxifies hydrogen peroxide in red algae, and the expansion of the catalase gene family in *Porphyra* and *Pyropia* (five genes) (*SI Appendix*, Table S21) compared with other red algae (one to two genes) could reflect the demand for detoxification of reactive oxygen species that cannot diffuse away from blades exposed by the falling tide to high light and air while they are still hydrated and photosynthetically active. Tocopherols prevent photooxidative damage of polyunsaturated fatty acids (45), and the *Porphyra* γ-tocopherol methyl transferase (*SI Appendix*, Table S22) catalyzes synthesis of α- and β-tocopherol. The isomer composition is unknown, but α-tocopherol has been identified in *Porphyra* blades and is considered the more potent antioxidant (46, 47). The 32 heat shock proteins (Hsp) in *Porphyra* indicate a possible expansion of the Hsp40 family (*SI Appendix*, Table S20), which are cochaperones of Hsp70 and play an important role in protein maturation and repair under normal and stressed conditions (48, 49).

Porphyra is frequently exposed to elevated intensities of UV radiation in the intertidal zone and shows remarkable tolerance to both UV-A and UV-B (50, 51). *Porphyra* has at least two strategies to protect photosynthesis and other key cellular processes from UV damage: mycosporine-like amino acids (MAAs) and circadian control over the timing of UV-sensitive processes.

MAAs act as "sunscreens" and comprise up to 1% of the dry weight of *Porphyra*, with the compound porphyra-334 being the major MAA (51). Four proteins—MysA, MysB, MysC, and MysD—support the biosynthesis of the MAA shinorine in cyanobacteria, such as *Nostoc* (52, 53), whereas MysD is replaced by a nonribosomal peptide synthase in *Anabaena* (Fig. 2). Cyanobacterial MysD shows a relaxed substrate specificity, with condensation of threonine instead of serine onto mycosporine-glycine to yield porphyra-334 (53). The *Porphyra* genome contains a gene encoding a MysA and MysB protein fusion that is also found in several other red algae that synthesize MAAs, as well as in some dinoflagellates, which were proposed to have acquired the still separate but neighboring genes from a cyanobacterium (54) (Fig. 2 and *SI Appendix*, Figs. S23 and S24). The presence of the *MysA–MysB* fusion in red algae suggests that dinoflagellates could have acquired these genes from red algae through secondary or serial endosymbiosis (54) (*SI Appendix*, Fig. S23). Moreover, *Porphyra* and related species contain a gene encoding a MysC–MysD fusion protein, and in the *Porphyra* genome the *MysA–MysB* and *MysC–MysD* fusion genes are next to each other but transcribed on opposite DNA strands with adjacent 5′-ends (Fig. 2). Although the *Chondrus* genome also contains clustered *MysA–MysB* and *MysC–MysD*, the fusion proteins are encoded on opposite strands with the 3′-ends of the genes adjacent. Conservation of the MAA gene cluster in *Porphyra* and *Chondrus* and the two gene-fusion events suggest that this arrangement provides a selective advantage and efficient MAA biosynthesis for red algae that experience high UV irradiance.

Developmental and abiotic stress responses are often associated with photoreceptors in eukaryotes. The plant circadian clock contains blue- and red-light photoreceptors, including cryptochromes (CRY) and phytochromes (PHY), to entrain the circadian clock (55, 56); these photoreceptors are also involved in other fundamental processes in plants, including growth and development. *Porphyra* does not appear to encode a PHY photoreceptor or a typical plant CRY photoreceptor, although it has maintained four genes of the CRY/photolyase family. The *Porphyra* CRY that is most like plant CRYs is similar to a DNA

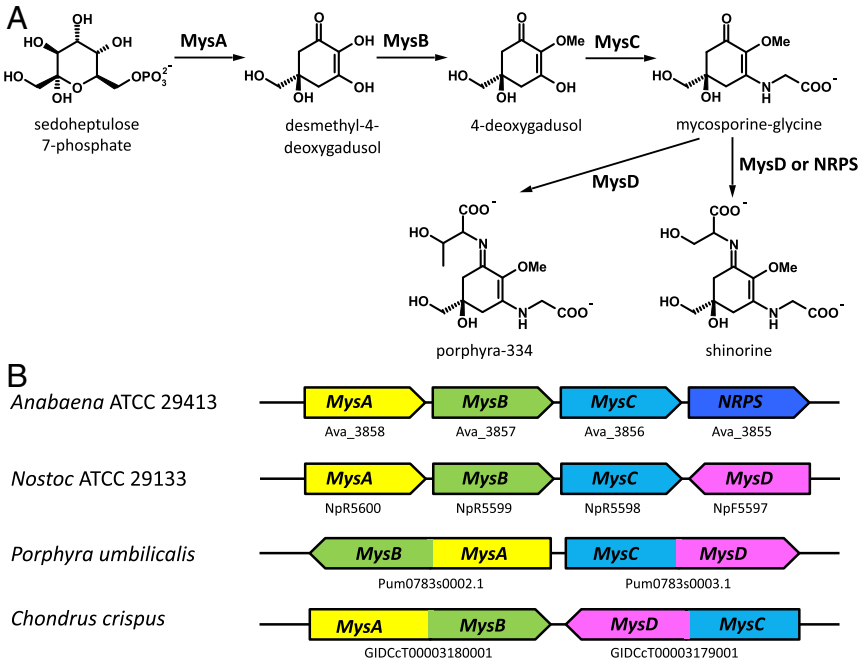

Fig. 2. Conserved gene clusters involved in MAA biosynthesis in *P. umbilicalis* and related red algae. (*A*) Biosynthetic pathway from sedoheptulose 7-phosphate to shinorine in cyanobacteria and proposed pathway to porphyra-334 in red algae. (*B*) Comparison of gene clusters and gene fusions in the cyanobacteria *Anabaena* and *Nostoc* and the red algae *P. umbilicalis* and *C. crispus*.

photolyase (PHR2) (*SI Appendix*, Fig. 27 and Table S26) (57). In contrast, other red algae seem to encode plant CRYs; however, these group closely to the cyclobutane pyrimidine dimer class III photolyases (58) in some cases (*SI Appendix*, Fig. S27). *Porphyra* also encodes a class II cyclobutane pyrimidine dimer photolyase (Pum0022s0035.1) as well as a CRY-DASH protein (59) (Pum2644s0001.1) in a separate subfamily of CRYs that displays DNA repair activity; these proteins are present from bacteria to vertebrates (59). In addition, there is an animal-like CRY (Pum0401s0002.1) encoded on the *Porphyra* genome (*SI Appendix*, Fig. S27). Animal-like CRYs could function as blue-light photoreceptors associated with the entrainment of the circadian clock, as they do in *Drosophila* and some other insects, or could even add an oscillatory component to the clock, as in mouse or humans (59). The animal-like CRY in *Porphyra* is closely affiliated with the cryptochrome photolyase family (e.g., *Ostreococcus tauri*, *Phaeodactylum tricornutum* in *SI Appendix*, Fig. S27), which has maintained photolyase activity (60, 61), in contrast to other plant and animal-like CRYs. Moreover, cryptochrome photolyase family CRY can affect transcriptional activity in a heterologous clock system (e.g., mammalian CRY) and control blue-light–dependent cellular processes, as found with insect or plant CRYs (60, 61). Similar to the *Chlamydomonas reinhardtii* animal-like CRY (aCRY), the Pum0401s0002.1 protein could sense blue light and possibly other light qualities, including red light (62, 63); this red-light sensing ability stems from the formation of the neutral radical form of the photoreceptor's flavin chromophore.

Signaling and Homeostasis. *Porphyra* must cope with significant osmotic and ionic stress in the intertidal zone. Several low molecular weight carbohydrates act as compatible solutes in red algae, including *Porphyra* (64–66). We found enzymes encoded on the *Porphyra* genome that support floridoside and isofloridoside synthesis, but no evidence for digeneaside synthesis (*SI Appendix*, Table S31). Taurine is a likely osmolyte in *Porphyra* (67), and we found homologs of enzymes, such as cysteine dioxygenase, implicated in metazoan taurine biosynthesis, which suggests an ancient origin for eukaryotic biosynthesis of this amino acid derivative (*SI Appendix*, Table S31). *Porphyra* also has a wide range of ion transporters, including an unusual class of Na^+/H^+ exchangers that are most similar to the NhaP class of transporters from α-Proteobacteria and are distinct from the Na^+/H^+ exchangers previously identified in eukaryotes. *Porphyra* has two P2C-type Na^+/K^+ ATPases and a P3A H^+-ATPase similar to those in land plants, suggesting *Porphyra* is able to energize its plasma membrane with either Na^+ or H^+ for secondary active transport, which could aid its survival in the intertidal zone (*SI Appendix*, Table S31). The *Porphyra* genome also contains a range of Ca^{2+}-permeable membrane channels that could play a role in osmotic stress signaling, including two homologs of OSCA (*SI Appendix*, Table S31), a recently identified Ca^{2+} channel in land plants (68); however, the Ca^{2+} sensor kinases through which *Porphyra* senses and responds to cytosolic Ca^{2+} elevations appear to be distinct from land plants and, indeed, from those of all other eukaryotes.

Land plants possess two expanded families of Ca^{2+} sensor kinases, the Ca^{2+}-dependent protein kinases (CDPKs) and the calcineurin B-like protein (CBL)-interacting protein kinases (CIPKs), which are activated through the binding of CBL (69, 70). The CIPKs have been characterized extensively in the green lineage (Viridiplantae), and they are present in other eukaryotes, including stramenopiles, haptophytes, and excavates, suggesting a likely origin early in eukaryotic evolution (71). Surprisingly, we found that genes encoding CIPKs and CBLs are absent from the *Porphyra* genome and from all other available red algal genomes and transcriptomes. The CDPKs are also absent from *Porphyra* and notably from other bangiophytes and florideophytes, but they are present in red algae in the SCRP clade (Fig. 3; compare *SI Appendix*, Fig. S11 and Table S32). Another class of Ca^{2+} sensor kinases, the Ca^{2+}/calmodulin-dependent protein kinases (CAMKs), which are important in both plants and animals, are also missing in *Porphyra*. This finding suggests that much of the extensive network of Ca^{2+} sensor kinases found in other eukaryotes is absent in the red algae (Fig. 3). Whereas the *Porphyra* genome encodes several proteins with domains similar

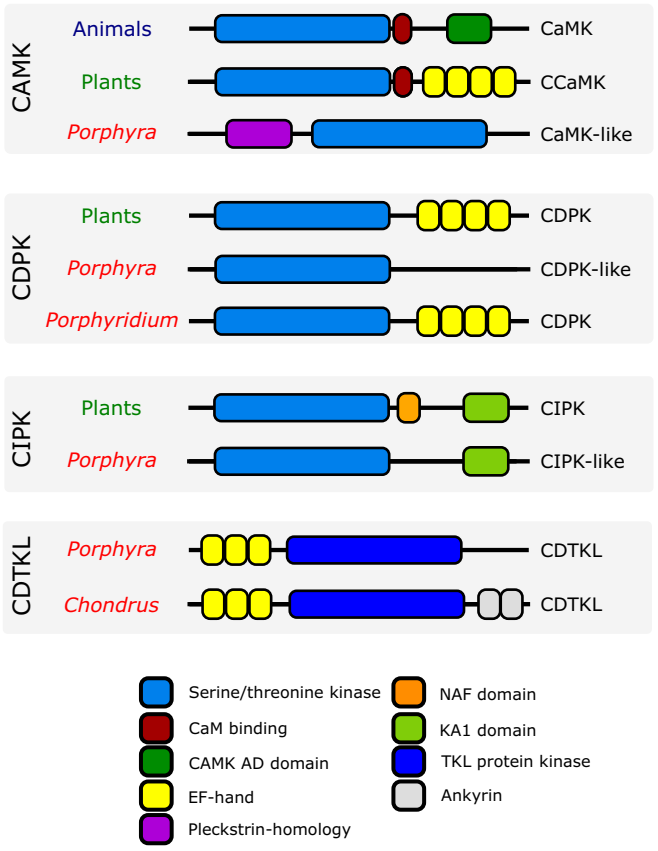

Fig. 3. Module structure and presence or absence of different Ca^{2+} sensor kinases in *Porphyra* and other eukaryotes: CAMK, CIPK, CDPK, CDTKL. The CDTKLs from *Porphyra* and other red algae represent a newly recognized family of protein kinases belonging to the TKL family that contains multiple Ca^{2+}-binding EF hands.

to the kinase domains of CAMKs, CDPKs, and CIPKs, there is no indication that these proteins are regulated by Ca^{2+} or Ca^{2+}-binding proteins. Instead, we found that *Porphyra* possesses a class of Ca^{2+} sensor kinases with two to three Ca^{2+}-binding EF-hand domains at the N terminus and a kinase domain at the C terminus (Fig. 3 and *SI Appendix*, Tables S32 and S33). The kinase domain from all known Ca^{2+} sensor kinases belongs to the CAMK group of kinases, whereas this uncharacterized protein belongs to the tyrosine kinase-like (TKL) class of kinases (72, 73). The genes encoding homologous proteins are present on many other red algal genomes, including *Chondrus*, *Cyanidioschyzon*, and *Galdieria*, but appear to be absent from all other characterized eukaryotic genomes. Hence, the Ca^{2+}-dependent TKLs (CDTKLs) represent a newly recognized class of Ca^{2+}-regulated kinases that appear to be unique to red algae (Fig. 3).

The sucrose nonfermenting-1–related kinase (SnRK) family of serine/threonine kinases plays important roles at the interface between metabolic and stress signaling from fungi (yeast) to land plants (74). There are 38 SnRKs divided into three subclasses (SnRK1, -2, -3) in *Arabidopsis*, and the *Porphyra* genome encodes 31 proteins with homology to the kinase domains of *Arabidopsis* SnRK proteins (*SI Appendix*, Fig. S34). Phylogenetic analysis places the *Porphyra* SnRK group in two clades that include *Arabidopsis* SnRK1 and SnRK3/CIPK, and a third expanded clade containing 19 unique members (*SI Appendix*, Fig. S34). Although no *Porphyra* SnRKs are most similar to the SnRK2 subfamily of *Arabidopsis*, several members of the SnRK3/CIPK clade contain motifs similar to the C-terminal domain II of SnRK2 (*SI Appendix*, Fig. S34), which mediates interaction with PP2C in the abscisic acid (ABA) signaling pathway (75, 76). Furthermore, there are homologs of several ABA biosynthetic genes in *Porphyra* (*SI Appendix*, Fig. S35*A*), and ABA synthesis and responses to exogenous ABA are reported for *Pyropia orbicularis* (77). Although highly speculative, the evidence raises the possibility that the SnRK family plays important roles in stress responses including ABA-mediated responses in *Porphyra*. Genomic data also support the presence of ethylene-mediated regulation in *Porphyra*, because the *Porphyra* genome contains genes encoding several proteins involved in ethylene biosynthesis (*SI Appendix*, Fig. S35*B*), and ethylene was detected and shown to induce stress responses in red algae (78).

The cell cycle is regulated by dimers of cyclins and cyclin-dependent kinases (CDKs). Except in *C. merolae*, where circadian rhythms and stress responses regulate the G_1/S transition (79), the red algal cell cycle is not well characterized. Results here, coupled with the earlier studies of *C. merolae*, show that regulation of the red algal cell cycle is similar to that of metazoans and plants (80, 81), except that we did not find cyclin D encoded in any red alga, including *Porphyra* (*SI Appendix*, Table S37). Cyclin Ds are well conserved in all other eukaryotes where they regulate the G_1/S transition. The mitotic-specific cyclin A (CYCA), a known cell-cycle progression regulator, might instead function in place of D-type cyclins in red algae. We found that the glaucophyte (Archaeplastida) *Cyanophora paradoxa* (2) also lacks a cyclin D homolog.

Plant Defense Genes. Bangiophyte pathogens include some oomycetes, viruses, and bacteria, and these organisms sometimes cause serious economic losses to nori aquaculture. Land plants detect pathogens via an array of cell surface pattern-recognition receptors (e.g., receptor-like kinases) and intracellular receptors (e.g., nucleotide-binding domain and leucine-rich repeat proteins) (82). We found no evidence for this higher plant type of pathogen detection, but two families of intracellular ligand-binding protein containing NB-ARC domains, and a family of potential extracellular receptors containing malectin and Ig-like fold-domains, were found in the multicellular red algal genomes (*SI Appendix*, Fig. S38). We also found a bangiophyte-specific family of at least three proteins harboring vWFA and C-type lectin domains (*SI Appendix*, Table S39); C-type lectin domains are involved in pathogen detection in some animals (83, 84).

Cell Walls. Red algae synthesize many unique polysaccharides, such as agars that contribute to their ecological success and are of significant interest to biotechnological and industrial applications. The cells of the blade of *Porphyra* spp. form a nonrigid cell wall that plays an important role in osmotic acclimation by allowing dramatic changes in cell volume without plasmolysis (85, 86). The skeletal component of the cell wall of blade cells is not cellulose but partially crystalline β-1,4-linked mannan in the outer cell wall, with a highly crystalline inner layer of β-1,3-linked xylan (87, 88). We discovered two glycosyltransferase (GT2) enzymes (Fig. 4 and *SI Appendix*, Table S40) in *Porphyra* that are closely related to plant cellulose synthase-like (CSL) CSLAs (mannan synthases) and CSLCs (xyloglucan synthases) and the green algal CSLs proposed to be implicated in mannan synthesis (89, 90). These *Porphyra* GT2 enzymes represent excellent candidates for mannan synthases, and their discovery in *Porphyra* suggests that both CSLA and mannan biosynthesis originated in the last-common ancestor of green and red algae, rather than in the Viridiplantae, as previously proposed (89). These findings suggest that mannans had an ancestral function in red algae, although it appears the Florideophyceae abandoned mannans for cellulose, whereas the Bangiophyceae alternate between a mannan/xylan-based cell wall in the gametophytes and a cellulose-based cell wall in the filamentous sporophytes. The *Porphyra* genome has two genes encoding glycosyl hydrolases (GH) of the GH113 family,

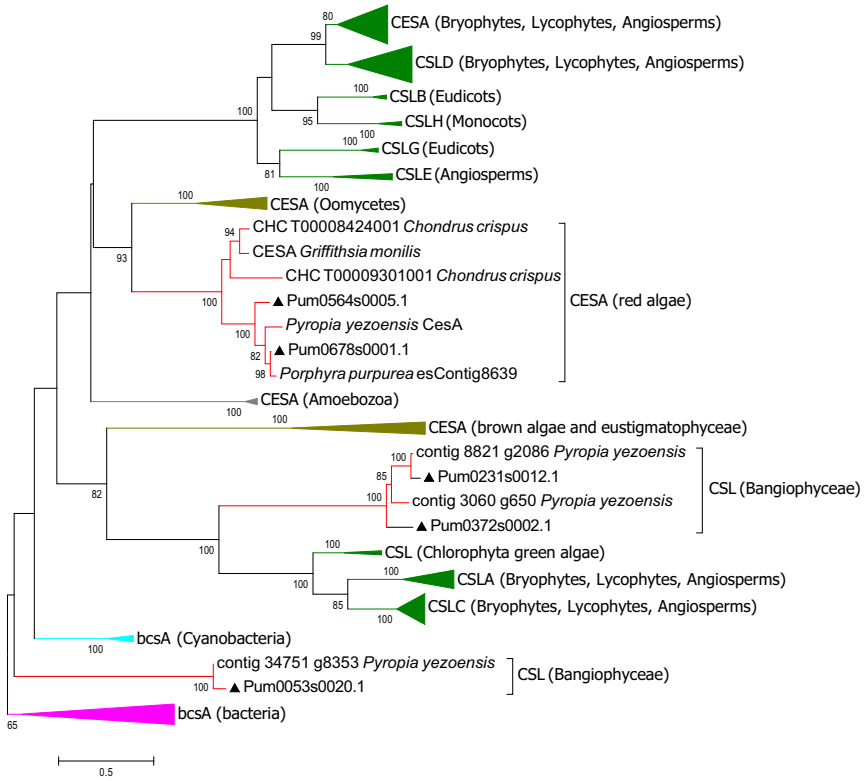

Fig. 4. Neighbor-joining phylogenetic tree of GT2 showing the positions of *Porphyra* sequences (dark triangles) and rooted on bacterial cellulose synthase (bcsA). Red algal cellulose synthases (CESA) cluster most closely with stramenopile oomycete CESAs, whereas bangiophyte CSL enzymes are sister to a clade of green plant CSLs that includes a mannan synthase. Two red algal GT2s are related to bcsA, likely because of horizontal gene transfer. Red algal sequences lie on red branches and triangles group sequences in Viridiplantae (green), stramenopiles (brown), Cyanobacteria (turquoise), Bacteria (pink), and Amoebozoa (gray). The proteins were aligned using MAFFT with the L-INS-I algorithm and the scoring matrix Blosum62 and then manually refined with BIOEDIT. The phylogenetic tree was calculated using maximum likelihood in MEGA 6.06. See *SI Appendix*, Table S42 for full names and GenBank accession numbers. Bootstrap values ≥ 65% are shown at nodes.

which are predicted to be β-mannanases, indicating that *Porphyra* has the enzymes required for both the biosynthesis and degradation of mannans present in the cell walls of the blade.

The extracellular matrix (ECM) component of the cell wall in *Porphyra* is a highly hydrated agar called porphyran that limits desiccation and contributes to the flexibility of the cell wall during osmotic stress (91) (*SI Appendix*, Figs. S41 and S45 and Table S40). Porphyran is mainly composed of porphyranobiose but also contains varying percentages of agarobiose (92). The *Porphyra* genome encodes a family 2 carbohydrate sulfotransferase (ST2) that may act as a L-galactose-6-O-sulfotransferase in the biosynthesis of porphyran (*SI Appendix*, Table S40). After *C. crispus*, the closest related homologs to this red algal sulfotransferase are found in metazoans (*Nematostella vectensis*, sea anemones) and the closest characterized enzymes are dermatan and chondroitin 4-O-sulfotransferases; similarly, the *Porphyra* GT7, after the *C. crispus* GT7, is most similar to metazoan GT7s (*Acropora digitifera*, staghorn corals) and the closest characterized enzyme is chondroitin sulfate *N*-acetylgalactosaminyltransferase 2 (*Homo sapiens*). These findings lend support to the ancestral nature of the biosynthesis of sulfated polysaccharides in the eukaryotes. Homologous carbohydrate sulfotransferases are indeed conserved at least in metazoans, brown algae, and red algae, so were most likely present in the last-common eukaryotic ancestor (91, 93). Genes encoding carbohydrate sulfotransferases are not found in sequenced genomes of terrestrial plants and freshwater algae, but marine angiosperms have, relatively recently, readapted to the marine environment by developing an ECM containing sulfated polysaccharides through an unknown mechanism of convergent evolution (94). This speaks to the critical role of sulfated ECMs in high-salinity environments (physiological saline and seawater). The *Porphyra* genome encodes three GH16 enzymes that form a clade with other red algae and with the agarases and porphyranases from marine bacteria (*SI Appendix*, Fig. S41). These enzymes likely function in cell wall remodeling and are unknown in unicellular red algae, which supports the possible involvement of bacteria in the evolution of multicellular algae (95).

Obtaining Nutrients at High Tide. We found that *Porphyra* has genes for Fe-uptake mechanisms that likely lend specificity and enable high-affinity assimilation during the narrow tidal window when blades are underwater (Fig. 5 and *SI Appendix*, Table S46). In addition to several putative Fe^{2+} transporters of the NRAMP and ZIP family proteins (which are typically broad-specificity divalent metal transporters) (96), the *Porphyra* genome encodes a high-affinity iron transport complex containing a permease (FTR1) and multicopper oxidase (FET3 in yeast/FOX1 in green algae) and members of the FEA and ISIP2a families. The latter are related algal-specific protein families containing secreted soluble proteins involved in iron assimilation and membrane-bound iron-uptake facilitators (97, 98). Although both iron-uptake strategies are found in microalgae, we were surprised to find FTR1 and FOX1 homologs in a macroalga, given the absence of this complex in higher plants and animals. Based on an analysis of phylogenetic distribution, FTR1 homologs are found in each of the three main Archaeplastida lineages (Viridiplantae, Glaucophyta, and Rhodophyta) but were lost after

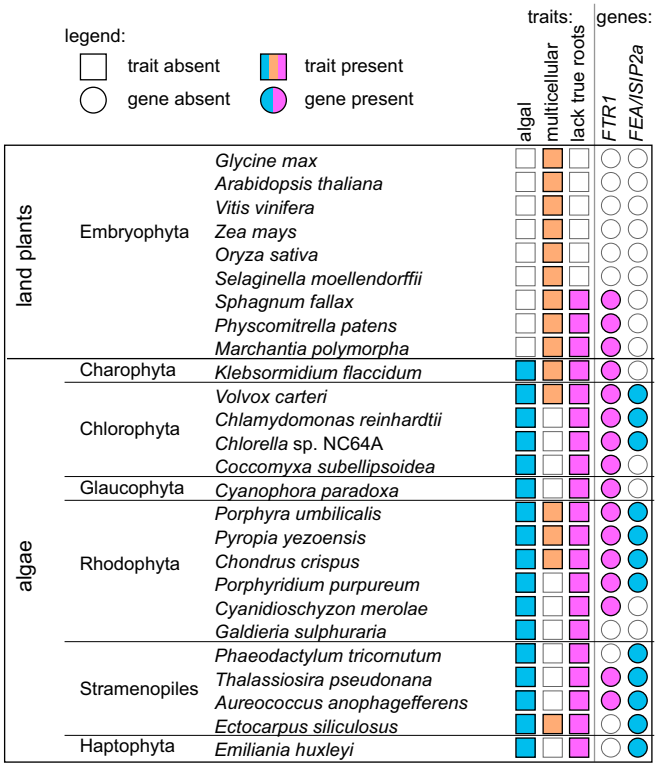

Fig. 5. Phylogenetic profile of *FTR1* and *FEA/ISIP2a* homologs in photosynthetic eukaryotes. Whether each organism is an alga, is multicellular, or lacks true roots is indicated with a colored square, and the presence of a *FTR1* or *FEA/ISIP2a* homolog in that organism is indicated with a colored circle. The same color is used to highlight the cooccurrence between a trait and a gene. Among sequenced algae and plants, *FTR1* homologs are only encoded in genomes of organisms that lack true roots, whereas *FEA/ISIP2a* homologs are only encoded by algal genomes. Multicellularity does not correlate with the presence of either protein family. The roots of *Selaginella moellendorffii* are morphologically and evolutionarily distinct, but share characteristics with true roots including a root cap and root hairs.

the transition to land when plants evolved true roots (Fig. 5). Other inorganic and organic nutrient uptake and conversion capabilities appear similar to those described for other red algae (e.g., Fig. 5 and *SI Appendix*, Fig. S51 and Tables S49, S52, and S53), except that the number of ammonium transporters (seven genes) (*SI Appendix*, Table S49) and intracellular copper transporters [P_{1B}-type ATPases; six genes (MTA1-6)] (*SI Appendix*, Fig. S47 and Table S46) is greater in *Porphyra*, perhaps reflecting the metabolic demands of life in the upper intertidal zone.

Porphyra ("laver") and related genera (i.e., *Pyropia*, "nori") are commercially important human foods based on their high mineral, protein, and vitamin content (99) (see Fig. 5 and *SI Appendix*, Figs. S51 and S59 and Tables S55 and S56 for related findings). No eukaryote synthesizes vitamin B_{12} (cobalamin), but *Porphyra* is a rich source of this organic micronutrient. Vitamin B_{12} is a required cofactor for B_{12}-dependent methionine synthase (METH), and *Porphyra* encodes both METH and METE, the latter a B_{12}-independent isoform of methionine synthase (100). Methionine synthase plays a vital role in linking the folate cycle, essential for DNA metabolism, and the methylation cycle responsible for production of S-adenosylmethionine, the universal methyl donor. The presence of METH implies that *Porphyra* takes up B_{12} from its abundant bacterial epiphytes (95), and we found that *Porphyra*, unlike other sequenced red algae, encodes both COBT and COBS. These enzymes are involved in remodeling cyanobacterial-produced pseudocobalamin (101) to cobalamin, the particular chemical variant of B_{12} that is bioactive for eukaryotic algae, and indeed humans (*SI Appendix*, Table S60). In *Chlamydomonas*, *METE* expression was found to be suppressed under environmentally relevant levels of heat stress, and survival was dependent upon thermally stable METH (102); the fact that *Porphyra* is frequently heat-stressed in its upper intertidal habitat might similarly explain why it retains both isoforms of this enzyme. Interestingly, the nutritional value of *Porphyra* appears to be directly related to the nutrient requirements for survival in a harsh habitat.

Summary

The red algae have long commanded attention because of the 1.2-Ga age of the multicellular bangiophytes, the unique intricacies of their life histories, and their economic importance, but genomic analysis of *Porphyra* sharpens our understanding of just how different the red algae are from other eukaryotes. Because the cytoskeleton is so central to growth, development, and the ability to respond to environmental signals, the paucity of cytoskeletal elements in *Porphyra* and other red algae is striking. Only kinesin motor proteins are universally present; dynein motors are absent, and most of the red algae, including *Porphyra*, appear to lack myosin. This minimal cytoskeleton offers a potential explanation for the extreme reduction in stature and complexity of red algae compared with most other multicellular lineages. The unique calcium-dependent signaling pathway further suggests that *Porphyra* may use distinctive mechanisms to sense and respond to its environment. Most bangiophytes including *Porphyra* live under harsh intertidal conditions. Our discovery of ancestral mechanisms of cell wall formation, an expanded array of UV/high-light/reactive oxygen species/thermal protection strategies, and a wealth of nutrient transporters encoded by the *Porphyra* genome helps to explain how these red algae have thrived for over a billion years in the pounding waves, baking sun, and drying winds of the high intertidal zone.

Methods

P. umbilicalis was isolated as an unialgal culture from a blade growing at Schoodic Point, Maine (44°20′1.68″ N; 68°3′29.14″ W) on April 3, 2008. This single isolate (103) was cloned by culture of progeny from its asexual neutral spores, followed by DNA extraction, and assembly of the genome from sequences produced using Illumina and Pac-Bio sequencing platforms (see *SI Appendix, Methods* for full details). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession no. MXAK00000000. The version described in this paper is version MXAK01000000.

ACKNOWLEDGMENTS. We are grateful to the anonymous reviewers and editor for their insightful and constructive suggestions. We thank Dr. Lilibeth Miranda, Charlotte C. T. Quigley, and Charlotte Royer (University of Maine) for their major assistance in maintaining cultures of the genomic strain during the project period; Drs. Joyce Longcore (University of Maine) and M. Blackwell (Louisiana State University) for discussions about fungi; Dr. Sarah Tepler Drobnitch for discussion about kelp sieve elements; and the many colleagues who provided useful discussion as participants in the *Porphyra* Research Coordination Network (supported by NSF RCN 0741907 [to S.H.B., A.R.G., and J.W.S.]), especially to Elisabeth Gantt (University of Maryland), an original co-PI of the NSF RSN and DOE JGI contract. The work conducted by the US Department of Energy (DOE) Joint Genome Institute, a DOE Office of Science User Facility, was supported by the Office of Science of the US DOE under Contract DE-AC02-05CH11231 (to S.H.B., E.G., A.R.G., and J.W.S.). Other major research support was provided by NSF 0929558 (to S.H.B. and A.R.G.); National Oceanic and Atmospheric Administration (NOAA) Contract NA060AR4170108 (to S.H.B.); German Research Foundation Grant Mi373/12-2 of FOR1261 (to M.M.); the French National Research Agency under IDEALG Grants ANR-10- BTBR-04-02 and 04-04 "Investissements d'avenir, Biotechnologies-Bioressources" (to J.C., E.F.-B., G.M., and S.M.D.); the New Hampshire Agricultural Experiment Station, Scientific Contribution No. 2694, supported by the US Department of Agriculture/National Institute of Food and Agriculture Hatch Project 1004051 (to A.S.K. and Y.C.); the Biotechnology and Biological Sciences Research Council (BBSRC BB/1013164/1) of the United Kingdom and European Union FP7 Marie Curie ITN Photo.Comm 317184 (to A.G.S. and K.E.H.); the Office of Biological and Environmental Research of the US DOE (C.E.B.-H.); the Connecticut Sea Grant College Program (R/A-38) and the NOAA National Marine Aquaculture Initiative

(C.Y.); the NIH MCB 1244593 (to H.V.G.); NSF and NIH Grants NSF-MCB 1412738, NIH P20GM103418, and NIH P20GM103638 (to B.J.S.C.O.); NSF Graduate Research Fellowship under Grant 1247393 (to B.N.S.); the UK Natural Environment Research Council IOF Pump-priming + scheme Grant NE/L013223/1 (to C.M.M.G. and Y.B.); NOAA Contract NA14OAR4170072 (to S.H.B.); and The Great Barrier Reef Foundation, Australian Research Council (DP150101875) and a University of Queensland Early Career Researcher Grant (to C.X.C.).

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